

# Spontan hipertansif sıçanların mide mukozasında lipid peroksidasyon ve apopitozis düzeyleri

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## Özet

**Amaç:** Spontan hipertansif sıçanlar esansiyel hipertansiyon için yaygın olarak kullanılan bir hayvan modelidir. Hipertansiyonun mide üzerindeki, gastrite bağlı kanama, mide mukozal kan akımı ve motilite değişiklikleri ile damar genişlemesi gibi çeşitli komplikasyonlarının bilinmesine karşın, şimdiye kadar hipertansif hayvanların mide mukozal apopitozisiyle ilgili araştırma yapılmamıştır. Bu çalışmada, spontan hipertansif sıçanların midelerinde lipid peroksidasyon düzeyleri ve apopitotik değişiklikler araştırıldı. **Gereç ve Yöntem:** Spontan hipertansif (n:10) ve normotansif Wistar Kyoto (n:10) ratlar kullanıldı. Mide mukozasında apopitozisin tespiti için terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling tekniği, lipid peroksidasyonunun göstergesi olarak thiobarbituric acid reactive substances düzeyleri ölçüldü. **Bulgular:** Spontan hipertansif sıçanlarda apopitotik hücre sayısında Wistar Kyoto sıçanlarla karşılaştırıldığında anlamlı bir artış saptanırken thiobarbituric acid reactive substances düzeyleri değişmemiştir. **Sonuç:** Sonuç olarak, bu çalışma ile ilk kez hipertansif sıçanların mide mukozasında apopitoziste bir artış gösterilmiştir ancak apopitozise yol açan mekanizmalar kesin değildir.

**Anahtar kelimeler:** Spontan hipertansif sıçan, apopitozis, mide dokusu, lipid peroksidasyon

## Abstract

### Lipid peroxidation and apoptosis levels in gastric tissue of spontaneously hypertensive rats

**Aim:** The spontaneously hypertensive rat is a widely used animal model for essential hypertension. Despite the recognized gastric complications of hypertension, such as bleeding from gastritis, gastric mucosal blood flow and motility and vascular ectasias until now no investigations have been conducted on gastric mucosal apoptosis in hypertensive animals. In this study, we investigated the lipid peroxidation and apoptotic changes in the stomachs on spontaneously hypertensive rats. **Methods:** Spontaneously hypertensive (n: 10) and normotensive Wistar Kyoto rats (n: 10) were used. Thiobarbituric acid reactive substances were determined as an indicator of lipid peroxidation and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling technique was used to detect apoptosis in gastric tissue. **Results:** While the number of apoptotic cells were increased significantly in spontaneously hypertensive rats, thiobarbituric acid reactive substances were not changed when compared Wistar Kyoto rats. **In conclusion,** data presented in the current study demonstrate the significant increase in gastric mucosal apoptosis in spontaneously hypertensive rats for the first time but underlying mechanisms of apoptosis are not clarified.

**Key words:** Spontaneously hypertensive rat; apoptosis; gastric tissue; lipid peroxidation,

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## Introduction

Arterial hypertension is a widespread health problem that affects many individuals worldwide (1). Cardiac hypertrophy and myocardial infarction, stroke, and renal failure are common clinical complications

associated with the development and progression of hypertension (2). Hypertension is a major vascular disorder that affects multiple tissues and organs. It is a known risk factor for coronary and cerebral arterial diseases and frequently causes fatal complications, namely heart attack and stroke (3). Spontaneously hypertensive rat (SHR) is a widely used animal model for human essential hypertension (4,5).

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There is increasing evidence indicating that oxidative stress plays an important role in the pathogenesis of arterial hypertension (6). The generation of reactive oxygen species (ROS) fluctuates in response to alterations of both external and internal environment and, in turn triggers specific signaling cascades, including mitogen-activated protein kinases, which determine cell survival or cell death (7). Apoptosis, a physiological mechanism of eliminating damaged or aged cells, also plays a major role in gastrointestinal epithelial cell turnover (8-10). In the stomach, mucosal surface epithelial cells are constantly exfoliating to the gastric lumen and completely replaced within 3-5 days under physiological conditions. Apoptosis has been reported to take place in all regions of the stomach with apoptotic cells occurring predominantly in the superficial parts of the gastric glands (11,12).

Dysregulation of apoptosis is a hallmark of numerous human pathologies including hypertension (13). Recently, several studies have shown that enhanced endothelial cell apoptosis promotes the disappearance of microvessels and leads to structural rarefaction in hypertensive rats (14,15). Another study indicates that higher inflammatory status in the important tissues in SHR might play a potential role in pathogenesis of hypertension and secondary organ complications (2).

Despite the recognized gastric complications of hypertension, such as bleeding from gastritis, gastric mucosal blood flow and motility and vascular ectasias (16,5) until now no investigations have been conducted on gastric mucosal apoptosis in hypertensive animals. The present study aimed to investigate possible changes in gastric mucosa due to hypertension in rats. Thus, thiobarbituric acid reactive substances (TBARS) were determined as an indicator of lipid peroxidation and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) technique was used to detect apoptosis in gastric tissue.

## Materials and Methods

### Preparation of animals

SHR (12–15 weeks of age) and age-matched normotensive Wistar Kyoto (WKY) rats (Harlan Laboratories, USA) were used in this study. The animals were housed at 23±2°C on a 12:12 h light–dark cycle and had free access to standard rat chow and drinking water. Systolic blood pressure (SBP) of all animals was monitored daily by tail-cuff method during the week before the experiments. The

experimental protocol was approved by the Animal Care and Usage Committee of Akdeniz University and was in accordance with the Declaration of Helsinki and International Association for the Study of Pain guidelines. Rats were divided into two groups, each consisting of 10 animals. Group 1; WKY rats, Group 2; SHR rats.

Rats were anesthetized with an intraperitoneal injection of thiopental sodium (80 mg/kg body weight). Their abdomens were opened by midline incision. The stomach was removed and opened at the lesser curvature. One part of the tissue was used for terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) technique to detect apoptosis in gastric tissue sections. Other parts of the tissues were used for TBARS assays, in order to detect lipid peroxidation.

### Measurement of lipid peroxidation

Lipid peroxidation of gastric tissues were estimated by the measurement of thiobarbituric acid reactive substances (TBARS) as described by Stocks and Dormandy using 1,1,3,3-tetraethoxypropane as standard (17). TBARS levels were determined by measuring absorbance at 532 nm after reaction with thiobarbituric acid in gastric tissues. The results were given as nmol/g protein.

### Determination of proteins

The amount of protein in the tissues was determined by using Lowry's method (18) referring to the albumin as standard.

### TUNEL Labelling

Apoptosis in mucosa and submucosa of stomach tissue was detected by enzymatic labeling of DNA strand breaks using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL). Paraffin sections of 5 µm thickness from the stomach tissues were cut and taken onto slides covered with poly-L-lysine and after drying the slides were left in the incubator at 45°C overnight, and at 60°C for 1 h. After deparaffinization and rehydration slides were washed twice in PBS for 5 min. Following the incubation of slides with the permeabilisation solution (0.1% Triton X-100 in 0.1% sodium citrate) for 8 min at 4°C and washing twice with PBS for 5 min, the labeling reaction was performed using 50 µl TUNEL reagent for each sample, except negative control, in which reagent without enzyme was added and incubated for 1 h at 37°C. Following PBS washings, slides were incubated with converter reagent for 30 min at 37°C. After

washing, color development for localization of cells containing labeled DNA strand breaks was performed by incubating the slides with Fast Red substrate solution for 10 min. TUNEL labeling was conducted using a Cell Death Detection kit (Roche; Mannheim, Germany) and performed according to the manufacturer's instructions. The apoptotic index was determined by counting a total of at least 100 nuclei subdivided in 6 fields chosen randomly at 400x magnification. Slides were examined by Zeiss Axioplan Microscope and photographs were taken with Spot Advanced Software.

### Statistical analysis

All values are given as means  $\pm$  SE. TBARS results were analyzed by One-way ANOVA. TUNEL results were analyzed by Student's t test.  $p < 0.05$  were considered significant.

### Results

SBP was significantly higher in SHR compared with WKY rats (SBPSHR  $189.6 \pm 1.4$  mmHg; SBPWKY  $132.6 \pm 2.8$  mmHg;  $p < 0.001$ ).

No significant changes were observed in SHR rats in TBARS levels when compared to WKY rats (SHR:  $497.2 \pm 53.1$ , WKY:  $530.8 \pm 84.8$ ). (Fig.1).

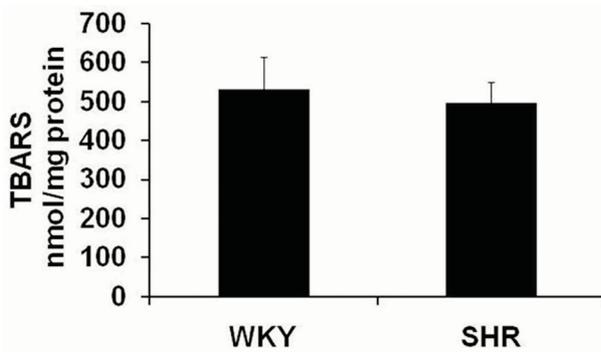


Figure 1: Gastric tissue TBARS levels on Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). No significant changes were observed between two group.

As represented in figure 2, the number of TUNEL-positive cells were significantly increased in SHR rats when compared to WKY rats on gastric mucosa. Figure 3, shows the apoptotic index of TUNEL positive cells. The number of apoptotic cells in SHR rats were significantly higher when compared to WKY rats (SHR:  $7.14 \pm 0.49$ , WKY:  $20.35 \pm 1.95$ ) ( $p < 0.05$ ).

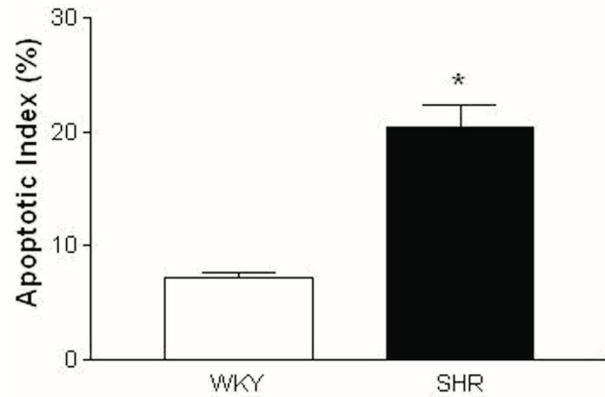


Figure 2: Graph shows the apoptotic index of TUNEL positive cells in Wistar Kyoto (WKY) and spontaneously hypertensive rats. The percentage of apoptotic cells were significantly higher in spontaneously hypertensive rats (SHR) when compared to Wistar Kyoto (WKY) rats. \* $p < 0.05$

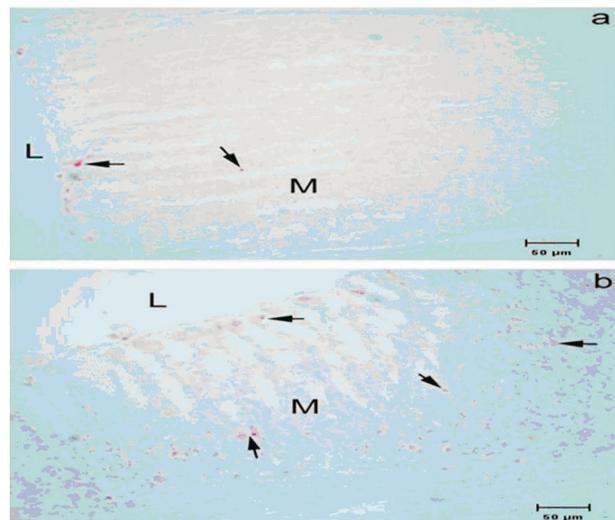


Figure 3: TUNEL assay in Wistar Kyoto (WKY) and spontaneously hypertensive rats. Arrows indicate the positive signals of apoptosis. (a) Wistar Kyoto (WKY) rats tissue sections with minimal apoptosis. (b) Spontaneously hypertensive rats tissue sections showing increased number of apoptotic cells in mukoza. L: Lumen M: Mucosa. (Original magnificationx50. Tunal reactions were determined by Fast Red as a chromagen).

### Discussion

The present study showed that gastric mucosal apoptosis was significantly increased in spontaneously hypertensive rats when compared to WKY rats. Spontaneously hypertensive rats were originally inbred from Wistar rats and their Wistar-Kyoto inbred non-hypertensive controls. These rats develop hypertension at about 4-6 weeks of age without physiological or surgical intervention (19). The importance of this model has been attributed to the similarity of its pathophysiology with essential hypertension in humans (20). Apoptosis is a highly

conserved process that plays an important role in controlling tissue development, homeostasis, and architecture. Dysregulation of apoptosis is a hallmark of numerous human pathologies including hypertension (13). Several reports have shown that, a role for apoptosis in the development of microvascular rarefaction in hypertension (15,14,21). Tsutsumi et al. demonstrated that DNA fragmentation resulting from spontaneous apoptosis occurs in the gastric mucosa of guinea pigs and that the fragmentation can be prevented by administration of caspase inhibitors (22). Several reports have shown that various gastric stressors (ethanol, hydrogen peroxide, and hydrochloric acid) and several stress conditions (water immersion and restraint stress, cold-restraint stress) are able to induce apoptosis in gastric mucosal cells (22-24). Apoptosis has also been shown to be induced following acute mucosal injury and during gastric ulcer healing (11). Our previous studies also showed that cold-restraint stress and sodium metabisulfite ingestion caused apoptosis in gastric mucosa (25,26). It is known that various gastric complications of hypertension, such as bleeding from gastritis, vascular ectasias and changes neuroendocrine cells function (5,16). However, no data are presently available gastric mucosal apoptosis in spontaneously hypertensive animals. The relationship between the development of hypertension and the increased bioavailability of reactive oxygen species, decreased antioxidant capacity or both has been demonstrated in many experimental models of hypertension, as well as in human hypertension (27,28). Lipid peroxidation is known to have deleterious effects on structure and functions of cell membrane (29). A substantially high level of endogenous lipid peroxidation is an indicator of biochemical disorder in cells, tissues, and organs, as well as an indicator of toxicological effect of some chemical toxins on living organisms (30). Recently, it is accepted that oxidative stress is an apoptosis inducer (31). The production of reactive oxygen species, in particular, has been associated with programmed cell death in many conditions such as stroke, inflammation, ischemia, lung edema, and neuro-degeneration (32-35). Data from the current study did not reveal any significant difference on SHR TBARS levels when compared to WKY rats. On the other hand, apoptosis is regulated by a complex cellular signaling network (36, 37). Several members of the protein kinase C (PKC) family serve as substrates for caspases and PKCdelta isozyme has

been intimately associated with DNA damage-induced apoptosis. It can act both upstream and downstream of caspases. In response to apoptotic stimuli, the full-length and the catalytic fragment of PKCdelta may translocate to distinct cellular compartments, including mitochondria and the nucleus, to reach their targets. Both activation and intracellular distribution of PKCdelta may have significant impact on apoptosis (36). Ren J, demonstrated that, protein carbonyl formation levels, an indicator of protein damage, increased markedly in SHR rats when compared to WKY rats on heart tissue (4).

In conclusion, data presented in the current study demonstrate the significant increase in gastric mucosal apoptosis in SHR rats for the first time but underlying mechanisms of apoptosis are not clarified. Protein carbonyl formation may play a role in SHR rat gastric apoptosis. However, further in-depth studies would be necessary to interpret the mechanisms underlying gastric apoptosis on SHR rats.

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